PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference ZRC-BT-003	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/IN 02/00216	International filing date *(day/month/year)* 01.11.2002	Priority date *(day/month/year)* 01.11.2002
International Patent Classification (IPC) or both national classification and IPC C12P21/02		
Applicant CADILA HEALTHCARE LIMITED et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
24.05.2004	27.01.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Morawetz, R Telephone No. +49 89 2399-8155

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IN 02/00216

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-35 as originally filed

Sequence listings part of the description, Pages

1-3 as originally filed

Claims, Numbers

1-21 as originally filed

Drawings, Sheets

1/10-10/10 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☒ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☒ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 21

   because:

   ☒ the said international application, or the said claims Nos. 21 relate to the following subject matter which does not require an international preliminary examination (specify):

   see separate sheet

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	2-10, 17, 18
	No:	Claims	1, 11-16, 19-21
Inventive step (IS)	Yes:	Claims	
	No:	Claims	2-10, 17, 18
Industrial applicability (IA)	Yes:	Claims	1-20
	No:	Claims	21: no opinion

2. Citations and explanations

see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step or industrial applicability

Claim 21 relates to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of this claim (Article 34(4)(a)(i) PCT).

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents, the numbering corresponds to the listing of the documents in the international search report:

   D1: GARCIA J N ET AL., BIOTECNOLOGIA APLICADA, LA HABANA, CU, vol. 12, no. 3, 1995, pages 152-155, XP002930567 ISSN: 1027-2852
   D2: WO 01/68827 A (PRASAD KOLLI SATYA NARAYANA ;SHANTHA BIOTECHNICS P LTD (IN); SRIRA) 20 September 2001 (2001-09-20)
   D3: EP-A-0 032 134 (BIOGEN NV) 15 July 1981 (1981-07-15)
   D4: DATABASE EMBL [Online] 29 July 1991 (1991-07-29), GOEDDEL D ET AL.,: "Homo sapiens interferon alpha-a (IFNA) gene, complete CDS." XP002240746 Database accession no. J00207
   D5: STREULI M ET AL., SCIENCE, vol. 209, 1980, pages 1343-1347
   D6: EP-A-1 236 800 (GENODYSSEE) 4 September 2002 (2002-09-04)
   D7: LEE N ET AL., JOURNAL OF INTERFERON AND CYTOKINE RESEARCH, MARY ANN LIEBERT, NEW YORK, NY, US, vol. 15, no. 4, April 1995 (1995-04), pages 341-349, XP001016231 ISSN: 1079-9907
   D8: LIU P T TA TUAN V VILLARETE L H., PROTEIN EXPRESSION AND PURIFICATION, ACADEMIC PRESS, US, vol. 22, August 2001 (2001-08), pages 381-387, XP002954670 ISSN: 1046-5928

2. Subject-matter of the application

   Present application relates to recombinant expression of human IFN alpha 2b in the methylotrophic yeast Pichia pastoris. The sequence of the human IFN alpha 2b used for recombinant expression (SEQ ID NO: 3) was derived from mRNA

isolated from human leucocytes and shown to have two point mutations, one at the 57th nucleotide position and the other at the 195th nucleotide position, compared to the published sequence (see GenBank Accession number J00207, D4 and D5) for human IFN alpha 2b. The protein encoded by SEQ ID NO: 3 is identical with the wild-type sequence of human IFN alpha 2b (see e.g. D4).

3. The present application does not satisfy the criterion set forth in **Article 33(2) PCT** because the subject-matter of claims 1, 11-16, 19-21 is not new in respect of prior art as defined in the regulations (**Rule 64(1)-(3) PCT**).

3.1. Claim 1 relates to a process for preparation and purification of recombinant human IFN alpha 2b.

D1 relates to high level expression of human IFN-alpha 2b in Pichia pastoris and discloses that the gene coding for human interferon-alpha-2b was integrated into the Pichia pastoris genome. The construction of the expression vector is summarized in Fig. 1. The recombinant IFN-alpha 2b was purified from the culture medium by harvesting the cells and recovering IFN alpha from the cells. The expression of the interferon gene was controlled by methanol-regulated alcohol oxidase (AOX1) promoter and an expression level of 400 mg of interferon per liter of culture was achieved under appropriate fermentation conditions (abstract). The molecule was purified to homogeneity and partially characterized in terms of biological activity and structural properties. Recombinant IFN alpha was found to be acetylated at the N-terminus in about 70% of the final product. According to D1 the amino terminal acetilation can be avoided by expressing the protein fused to another protein, for example ubiquitin, and processing the hybrid protein with specific hydrolases.

D1 is thus considered to anticipate the subject-matter of claims 1 and 11-16.

3.2. D6 relates to nucleic acids encoding IFN-alpha 2b containing SNPs and discloses (page 13, line 45 - page 16, line 13; example 10) pharmaceutical compositions comprising purified alpha 2b as well as methods of treatment using purified IFN-alpha 2b and thus anticipates the subject-matter of claims 19-21.
Applicant's attention is drawn to the fact that the product of a novel process is by no means also necessarily novel. As a matter of fact any pharmaceutical composition comprising a (any) human interferon alpha 2b is considered to fall within the scope of claim 19. Such a composition has also, at least implicitly, been

disclosed by D7 (page 341, left hand column, 2nd paragraph).

3.3. The subject-matter of claims 2-10, 17, 18 appears to be novel in view of the available prior art.

4. The present application does not satisfy the criterion set forth in **Article 33(3) PCT** because the subject-matter of claims 2-10, 17, 18 does not involve an inventive step as defined in the regulations (**Rule 65 (1)-(2) PCT**).

4.1. Claim 2 relates to a process for preparation and purification of recombinant human IFN alpha 2b in Pichia pastoris as claimed in claim 1 wherein said human IFN alpha 2b gene comprises the sequence of SEQ ID NO: 3.

4.2. The nucleotide sequence of human IFN alpha 2b (see D4, D5) as well as its recombinant expression in yeast in general and in Pichia pastoris in particular has been disclosed in the prior art (see D1-D3, D6). Thus D4 (GenBank Accession number J00207) discloses the complete coding sequence of human IFN alpha 2b which shows 99.598% identity (99.598% ungapped) in 498 nt overlap (1-498:580-1077) with SEQ ID NO: 3 and differs from the sequence of SEQ ID NO: 3 only at positions 57 and 195. D1 discloses high level expression of human IFN-alpha 2b in Pichia pastoris. D2 discloses a process for the production of physiologically-active human interferon alpha from genetically engineered yeast such as Pichia pastoris, D3 discloses recombinant production of IFN alpha 2b in yeasts.
D6 discloses (page 4, line 47-54; example 6) expression of natural wild-type IFN alpha 2b (GenBank Accession number J00207) in Pichia pastoris. The sequence encoding the natural wild-type IFN alpha 2 b is amplified by PCR and the PCR product is inserted in expression vector pPicZα-TOPO which allows expression under the control of the hybrid promoter AOX1 inducible by methanol. Due to the presence of the signal peptide sequence of the "alpha factor", upstream of the coding sequence, the protein is secreted by the yeasts in the culture medium and the alpha factor is naturally cleaved during the processing. The protein is purified from the obtained supernatant and purity of the protein is estimated on SDS/PAGE gel. Various activities of the protein are tested in examples 7-10. The protein of D6 is apparently not acetylated.

The subject-matter of claim 2 differs from this prior art in that the sequence which is used to express human IFN alpha 2b differs from the wild-type IFN alpha 2 b

sequence (GenBank Accession number J00207) employed in the prior art in two positions, while encoding the same protein.

The problem to be solved by the present application can thus be seen in the provision of alternative means for the recombinant production of human IFN alpha 2b.

The problem has been solved with the provision of the process according to claim 2.

The solution proposed in claim 2 of the present application cannot be considered as involving an inventive step (Article 33(3) PCT) for the following reasons.

The sequence of present application (SEQ ID NO: 3) has been cloned from human leucocytes and the two differences observed with respect to the wild-type sequence are considered to represent mere single nucleotide polymorphisms (SNPs) which are known to occur with a certain frequency in all human genes in general and in the gene encoding human IFN alpha 2b in particular. Thus, D6 discloses (page 3, lines 4-6) the identification of 9 SNPs in the nucleotide sequence of the reference wild-type IFN alpha 2b gene.

It can thus not be argued that SEQ ID NO: 3 has been codon optimized to be better expressed in Pichia pastoris. It can also not be argued that the use of this particular sequence results in particular high levels of recombinant protein since according to examples 8-11 of present application the protein yield was 200mg/l, whereas D1, which uses the wild-type sequence, achieved 400 mg/l (page 153, right hand column, 4th paragraph). According to present application (Example 1) 550-600 mg/l could be achieved under particular conditions, these conditions are however not reflected in the present set of claims.

The provision of a process wherein the human IFN alpha 2b gene comprises SEQ ID NO: 3 has to be considered an arbitrary choice form all the possible solutions, i.e. processes based on all other human IFN alpha 2b sequences comprising SNPs, which is devoid of an inventive activity, in particular in view of the fact that no surprising technical effect is connected to it.

4.3. Dependent claims 3-10, 17 and 18 do not contain any features which, in combination with the features of any claim to which they refer, meet the require-

ments of the PCT in respect of inventive step, the reasons being as follows:

Human IFN alpha 2b is known to be expressed in leucocytes and has been cloned from human leucocytes before (see e.g. D5, D7) rendering the subject-matter of claim 3 obvious.

Cloning of human IFN alpha 2b by PCR is known from D1 and D6, rendering the subject-matter of claim 4 obvious.

The use of Pichia as a host for the recombinant expression of human IFN alpha 2b in combination with the AOX promoter and the alpha mat signal sequence as well as purification of the recombinant IFN is known from D6 rendering the subject-matter of claims 5-10, 17 and 18 obvious.